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PRINCIPAL INVESTIGATOR:
Erika J. Mitchell, M.D.

CONTRACTING ORGANIZATION:
Vanderbilt University Medical Center
Nashville, TN 37232

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Erika J. Mitchell
Email: erika.j.mitchell@vanderbilt.edu

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Vanderbilt University Medical Center
Medical Center East, S. Tower
Suite 4200
Nashville, TN 37232

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14. ABSTRACT

We build on our earlier findings of three potential contributing genetic factors (ADRB2, TLR4, CFH)in the development of heterotopic ossification (HO). Demographic and environmental data for 1313 patients were compiled in addition to radiographic findings of HO in the same cohort. Statistical analyses were performed to determine associations between ISS, head AIS, ICU days, days on ventilator support, race and gender on the development of HO.

In addition, we have begun extraction on another 1161 patient specimens to add genetic data to the 2426 specimens already extracted in our repository. Along with the 36 SNPs we planned to investigate in our original proposal, we will add an additional 60 SNPs to include more pathways recently identified in the literature and that are associated with our initial findings.

15. SUBJECT TERMS
Heterotopic Ossification, Single Nucleotide Polymorphysms, Genetics, Fracture Healing

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INTRODUCTION

The interplay of environmental influences on an individual's genetic template is complex. Clearly, individuals react to environmental triggers based upon genetic programming even though the response may be mitigated by yet other environmental factors. This is the basis for understanding the spectrum of severity in genetic diseases and the ability to treat such diseases by altering the physiologic environment.

Last year, we were able to demonstrate an association of three single nucleotide polymorphisms (SNPs) with risk for the development of heterotopic ossification (HO) after traumatic fracture. A minor allele for the B2 adrenergic receptor was associated with an increased risk of forming HO. Minor alleles in polymorphisms for Toll-Like Receptor 4 and Complement Factor H on the other hand appear to be protective.

We continued to build off of this pilot data to determine if factors such as Injury Severity Score (ISS), Head Abbreviated Injury Scale (HAIS), ventilator days, and demographics correlate with the development of HO. In addition, we are continuing the extraction of DNA from 2275 more patients to bring us to a total of 3587 specimens for genotyping. We will add to the data pool an additional 30 candidate genes to broaden the possible genetic factors contributing to HO formation.

BODY

Aim 1: To examine the relationship of genetics to the Heterotopic Ossification phenotype

To date, we have accrued 6000 patients with specimens in our genetics repository. We currently have 2426 specimens that have undergone extraction and have begun extraction on another 1161 specimens. Genotyping will continue and we now plan a total of 96 SNPs. This is an increase from our original proposal of 36 SNPs. We have made this change secondary to a decrease in cost of genotyping and the knowledge gained from our pilot study and current literature. We have generated a list of candidate genes that include pathways associated with the three SNPs we identified earlier as well as recent findings in genetic forms of diseases that are associated with excessive bone growth such as Fibrodysplasia Ossificans Progressiva and Hereditary Exostoses.(1-3)

Aim2: To determine the environmental effect (ie. injury severity, traumatic brain injury, medications) on phenotypic expression

We compiled data on 1313 patients including demographics, injury severity score (ISS), head abbreviated injury severity score (AIS head), ICU days, and ventilator days. We are continuing to compile sepsis data and medication data to also correlate with the formation of heterotopic ossification. We ran a bivariate analysis as well as a logistic regression analysis on the data which is summarized in the Results section. The analysis was performed by a faculty level statistician in the Department of Biostatistics at Vanderbilt University.

Aim3: To determine clinical biomarkers which predict the HO phenotype

The data has been captured for the 6000 patients with specimens in the repository. We will organize this data once we are certain which patients in the database have been successfully genotyped and meet the inclusion criteria.

KEY RESEARCH ACCOMPLISHMENTS

- DNA extraction performed on 2426 specimens
- Data has been compiled for 1313 patients including:
    - ISS
    - Head AIS
    - Number ICU days
    - Ventilator Days
    - Ventilator Assisted Pneumonia
    - Age
    - Race
    - Gender
- Manuscript submitted to Journal of Orthopaedic Trauma
    - Accepted pending minor revision
- Presented at the OTA Annual Meeting, San Diego CA 2009
- Presented at the Extremity War Injuries Symposium, Washington DC 2010

REPORTABLE OUTCOMES

Demographics

Gender (Data available for 1299 patients)

Male 964
Female 335

Race (Data available for 1301 patients)

White 1037
Black 158
Hispanic 91
Other 15

Bivariate analysis of the data is summarized in Table 1.

	HO (1=y, 0=n)	N	Mean	STD	P value
AGE	0	1181	41.78	18.51	0.443
	1	113	42.14	16.17	
ISS	0	1182	26.02	12.58	.0223
	1	116	28.76	11.56	
AIS Head	0	1183	2.35	1.92	<.0001
	1	116	1.70	1.77	
Hosp Days	0	1183	11.78	12.52	<.0001
	1	116	18.16	16.86	
ICU Days	0	1145	5.49	7.09	<.0001
	1	110	7.43	6.11	
Vent Days	0	1145	4.68	7.00	<.0001
	1	111	6.15	5.96	

Logistic regression analysis of effect with the response variable of HO is summarized in Table 2.

Effect	Odds Ratio	P value
Age	0.97	0.327
ISS	**1.15**	0.0003
AIS Head	**0.74**	<0.0001
ICU Days	1.08	0.135
Vent Days	0.94	0.252

CONCLUSION

We have previously identified three single nucleotide polymorphisms associated with the formation of HO in 1095 patients. In this same cohort, we have also now compiled data on possible contributing factors to the formation of HO.

Two factors were noted to be statistically significant. Surprisingly, the abbreviated injury severity score for head injury was not associated with an increased incidence of HO in this population. Instead, the odds ratio for head injury and associated HO was 0.75. Injury severity score was however positively correlated with an odds ratio of 1.15. Further statistical analysis needs to be performed to evaluate differential effects of these factors since head injury scores are included in ISS and both may be associated with ICU and ventilator days.

Gender was associated with risk with female gender actually more likely to develop HO (p=.0433) however the data was skewed due to a significantly greater number of male patients (M=887, F=296). Race associations were difficult to assess because of the disproportionately high number of Caucasians (n=964) but statistically, Caucasian race was associated with increased risk of HO (Odds ratio 1.23, p=0.011).

In addition to further statistical analysis of the data, we have begun extraction and will be generating chips to analyze for the new candidate SNPs in addition to the original list of 36 SNPs. Appendix 1 is a list of potential candidates based on literature review.(1-12) The SNPs for each gene of interest were identified using Phase III of the International HapMap Project (www.HapMap.org) to identify independent regions of each gene to avoid inclusion of SNPs that overlap.

Our initial findings correlating environmental and demographic factors with the development of HO are somewhat surprising. We do not find correlation of HO with severity of head injury. One of our initial considerations in the high incidence of HO in the combat amputee population was the potential prevalence of occult head injury incurred by the blast mechanism of many of these injuries. Clearly, we would like to use all of the data we have available in the 6000 patients to be more definitive and accurate about our findings both genetically and epidemiologically.

Reference List

(1) Faiyaz-Ul-Haque M, Ahmad W, Zaidi SH, Hussain S, Haque S, Ahmad M, et al. Novel mutations in the EXT1 gene in two consanguineous families affected with multiple hereditary exostoses (familial osteochondromatosis). Clin Genet 2004 Aug;66(2):144-51.

(2) Hilton MJ, Gutierrez L, Martinez DA, Wells DE. EXT1 regulates chondrocyte proliferation and differentiation during endochondral bone development. Bone 2005 Mar;36(3):379-86.

(3) Kaplan FS, Xu M, Seemann P, Connor JM, Glaser DL, Carroll L, et al. Classic and atypical fibrodysplasia ossificans progressiva (FOP) phenotypes are caused by mutations in the bone morphogenetic protein (BMP) type I receptor ACVR1. Hum Mutat 2009 Mar;30(3):379-90.

(4) Baek K, Bloomfield SA. Beta-adrenergic blockade and leptin replacement effectively mitigate disuse bone loss. J Bone Miner Res 2009 May;24(5):792-9.

(5) Chen HY, Tsai HD, Chen WC, Wu JY, Tsai FJ, Tsai CH. Relation of polymorphism in the promotor region for the human osteocalcin gene to bone mineral density and occurrence of osteoporosis in postmenopausal Chinese women in Taiwan. J Clin Lab Anal 2001;15(5):251-5.

(6) Elefteriou F, Takeda S, Ebihara K, Magre J, Patano N, Kim CA, et al. Serum leptin level is a regulator of bone mass. Proc Natl Acad Sci U S A 2004 Mar 2;101(9):3258-63.

(7) Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, et al. Complement factor H variant increases the risk of age-related macular degeneration. Science 2005 Apr 15;308(5720):419-21.

(8) Lowik CW, van Bezooijen RL. Wnt signaling is involved in the inhibitory action of sclerostin on BMP-stimulated bone formation. J Musculoskelet Neuronal Interact 2006 Oct;6(4):357.

(9) Mundy GR, Elefteriou F. Boning up on ephrin signaling. Cell 2006 Aug 11;126(3):441-3.

(10) Tsiridis E, Giannoudis PV. Transcriptomics and proteomics: advancing the understanding of genetic basis of fracture healing. Injury 2006 Apr;37 Suppl 1:S13-S19.

(11) Turker S, Karatosun V, Gunal I. Beta-blockers increase bone mineral density. Clin Orthop Relat Res 2006 Feb;443:73-4.

(12) Willing MC, Torner JC, Burns TL, Janz KF, Marshall T, Gilmore J, et al. Gene polymorphisms, bone mineral density and bone mineral content in young children: the Iowa Bone Development Study. Osteoporos Int 2003 Aug;14(8):650-8.

Appendix

List of additional candidate SNPs to be tested

CFH
- rs12038333
- rs11582939
- rs6677604
- rs1329428
- rs12405238
- rs800292
- rs10922096
- rs3766404
- rs7524776
- rs2284664
- rs424535
- rs6695321
- rs419137

TLR4
- rs1927911
- rs5030717
- rs11536878
- rs5030728

ADRB2
- rs1042717
- rs1042713
- rs1801704

LRP5
- rs607887
- rs7111370
- s12417792
- rs12417014
- rs3736228
- rs638076
- rs901823
- rs901824
- rs3781579
- rs632605

CRP
- rs1130864
- rs1205

BMP2
- rs235764
- rs235767
- rs1005464
- rs7270163
- rs3178250
- rs170986

BMP4
- rs17563
- rs762642

ACVR1
- rs1146035
- rs12987698
- rs12997
- rs17182166
- rs3820742
- rs10497189
- rs10933441
- rs10497191
- rs10497192
- rs13398650
- rs10933443

IL6
- rs17852649
- rs16829209
- rs2502450
- rs11577442
- rs11578307
- rs17852648
- rs11249201
- rs7418238
- rs3795300
- rs4486393
- rs3795302

IL1
- rs11686153
- rs2287041
- rs11903354
- rs12987900
- rs955754
- rs12474258
- rs11123914
- rs17637748
- rs11123913
- rs11692230
- rs13014084
- rs17026782
- rs1997502
- rs10167431
- rs6752589
- rs6752467

RANKL
- rs1038434
- rs3742257
- rs931273
- rs12585229

TNFα
- rs3093553

SOST
- rs865429

rs numbers TBD for:
RANK
GNAS
EXT1
EXT